Synthesis and Evaluation of Tri-cyclic Alkaloid-like Compounds as Anticancer Agents

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Declaration / Certificate of authorship and originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

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Dedication

I dedicate this thesis to my late beautiful Aunty, Wendy Xu, who was battling cancer. Her resilience and courage to fight this disease has set new standards for me and she continues to inspire me every day. Every day I go into the laboratory hoping that one day we can eradicate this disease.

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List of Abbreviations

Å	Angstrom
δ	Delta (Chemical shift in parts per million)
µM	micro-molar
V_{max}	Maximum absorbance
$[\alpha]_D^T$	Specific rotation for a Na lamp at 589nm
$[M+H]^+$	Protonated molecular ion
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
br s	Broad singlet
d	Doublet
ºC	Degrees Celsius
Da	Dalton
DCM	Dichloromethane
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DMAD	Dimethyl acetylenedicarboxylate
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
dq	Doublet of quartets
dt	Doublet of triplets
EDTA	Ethylenediaminetetra acetic acid
ER	Oestrogen receptor
EtOAc	Ethyl acetate
Equiv.	Equivalents

FTIR	Fourier transform infrared spectroscopy
g	gram
HER2	Human epidermal growth factor receptor 2
HSQC	Heteronuclear single quantum coherence
HRMS	High resolution mass spectrometry
Hz	Hertz
IC_{50}	The half maximal inhibitory concentration
J	Coupling constant (NMR)
MeOH	Methanol
m	Multiplet
mL	millilitre
mmol	milli mole
m.p	Melting point
MS	Mass spectrometry
$NaBH_4$	Sodium borohydride
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
$Na(OAc)_3BH$	Sodium triacetoxyborohydride
^1H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon nuclear magnetic resonance
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser effect spectroscopy
PBS	Phosphate buffered saline
PI	Propidium iodide
ppm	Parts per million

PR	Progesterone receptor
PSA	Polar surface area
q	Quartet
R_f	Retention factor
RPMI	Roswell park memorial institute medium
r.t.	Room temperature
s	Singlet
t	Triplet
td	Triplet of doublet
TLC	Thin layer chromatography

Publications from this Thesis

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Abstract

As part of our ongoing research for anticancer agents, the synthesis of a library of tricyclic compounds using the Bridging Ritter reaction has been described in Chapter 2. Tricyclic imines **82a-c** and amines (**90a-b, 91a-b**) were synthesised in good yields (> 70%). Eleven alkaloid-like compounds were successfully synthesised.

Encouraged by the synthetic success described in Chapter 2, preparation of tricyclic caryophyllene derived alkaloid-like compounds was undertaken to provide new leads as described in Chapter 3. The synthesis focused on the use of β-caryophyllene **96** and caryophyllene monoepoxide **117** as key starting materials in the Ritter reaction. Treating **96** and **117** with various nitriles under strong acidic conditions afforded optically active tricyclic amides of caryolane **119a-i** and clovane **120a-d** skeletons. The formation of these skeletons proceeded *via* the acid catalysed Wagner-Meerwein rearrangement. The rations of the two structures depended on the reactivity of the nitriles that led to the more kinetically stable of the two skeletons. Caryolane **119c** and clovane **120c** and **126c** were further used to generate complex alkaloid-like compounds through sequential amide cleavage and reductive alkylation. A total of 30 caryophyllene derived alkaloid-like compounds were successfully obtained and subjected to antiproliferative assays.

Chapter 4 describes the biological activities of the synthesised compounds described in Chapter 2 and 3. In-house *in vitro* biological assays were used to assess the cytotoxicities of the synthesised compounds on breast cancer cell lines MCF-7 (ER+) as well as MDA-MB-231 (triple negative). Compound **63** was selected by the US National Cancer Institute (NCI) for their standard cytotoxicity screening program. It was shown to have significant anti-cancer activities with IC_{50} in the μM range, across seven cancer types. The anti-cancer activities of **82c** were found to be selective towards the aggressive and more challenging to treat triple negative (MDA-MB-231) cell line while exhibiting no antiproliferative activities towards the

MCF-7 cells at the highest concentration tested (50 μM). The IC_{50} of compound **82c** was determined to be 7.9 μM for the MDA-MB-231 cell line. Furthermore, **82c** arrested cell cycle at the G_2/M phase and induced apoptosis in a dose-dependent manner. Cytotoxicities of compounds **63** and **82c** were tested against noncancerous mammalian cells (Vero cell line) and found to be approximately eight folds more selective towards MDA-MB-231 than the Vero cell line.

From caryophyllene-derived compounds, eight compounds effectively decreased the proliferation and viability of MDA-MB-231, with observed IC_{50} values ranging from 3.0 – 55.3 μM. Amongst the eight, **119c**, **120c**, and **126a** were most active and selective towards the more aggressive triple negative (MDA-MB-231) over the MCF-7 cells. Furthermore, compounds **119c**, **120c**, and **126a** also altered the distribution of cells throughout the cell cycle, as well as the ability to induce apoptosis in the MDA-MB-231 cells. This observed selectivity towards the harder to treat triple negative breast cancer cells make these compounds more ideal drug candidates for further development.